THE GROWTH OF LARVAE OF TIPULA OLERACEA LINNAEUS, 1758 (DIPTERA, TIPULIDAE)

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Abstract

The growth of larvae of *T. oleracea* Linnaeus, 1758, was studied under laboratory conditions. The body length, the head capsule length as well as the head capsule width were taken as a measure of growth. In addition, some preliminary observations on *T. paludosa* Meigen, 1830, are given. The results are discussed in the context of the evolutionary relationships between the species of the subgenus *Tipula.*

Introduction

The present study was carried out in order to establish the evolutionary relationships between the species of the subgenus *Tipula,* one of the several subgenera of the genus *Tipula* Linnaeus, 1758 (Diptera, Tipulidae). Originally, this subdivision was based on wing markings and wing venation (Edwards, 1931). Subsequently it was supplemented by characteristics of the male hypopygium (Mannheims, 1952—1968; Savtshenko, 1961, 1964; Theowald, 1973) as well as larval and pupal characters (Theowald, 1957, 1967).

The aim of the present and forthcoming studies is to add biological and morphometric data to the already available systematic criteria in order to establish more precisely the evolutionary relationships between the species.

The species of the subgenus *Tipula* occur in the Palaearctic as well as in the Ethiopian region. The Palaearctic species may be divided into two groups on the basis of their geographical distribution. Three species (*T. paludosa* Meigen, 1830, *T. oleracea* Linnaeus, 1758, and *T. czizeki* De Jong, 1925) are wide-spread in Europa, and even throughout the Palaearctic region into Japan (*T. czizeki*). The other seven species are more or less restricted to the Mediterranean in a broad sense: *T. mediterranea* Lackschewitz, 1930 (Western Mediterranean), *T. italica* Lackschewitz, 1930 (eastern Mediterranean), *T. orientalis* Lackschewitz, 1930 (eastern Mediterranean and the Middle East), *T. kleinschmidti* Mannheims, 1950 (Spain), *T. hungarica* Lackschewitz, 1930 (Austria, only three specimens known), *T. plumbea* Fabricius, 1781 (southern France, Sardinia and Greece) and *T. atlantica* Mannheims, 1962 (Madeira, only three males known) (Mannheims, 1952, 1962; Den Hollander, 1975).

In the Netherlands and surrounding region only three species of the subgenus *Tipula* occur. These species, and especially *T. paludosa,* are very important economically as the larvae can cause serious damage to crops. Most of the species mentioned above have two generations per year, one flying in spring, the other in autumn. However, *T. paludosa, T. italica* and *T. czizeki* only show an autumn flying period. The number of genera-

tions per year, however, seems to be variable as Simova (pers. comm.) mentions a spring as well as an autumn generation for *T. czizeki* in Yugoslavia.

In nature several species occur together at the same time and in the same habitats. Moreover, the males of these species do not show clear courtship behaviour but try to copulate by grasping every tipulid female, independent of the species to which it belongs. So, under laboratory conditions, cross-matings can be observed which produce fertile eggs, and even larvae and adults can be obtained from these crosses, e.g. between *T. paludosa* and *T. czizeki, T. oleracea* and *T. czizeki,* and also between *T. oleracea* and *T. paludosa* (Hemmingsen & Theisen, 1956; Den Hollander, unpubl.). Moreover, among the descendants of a *T. czizeki* female caught in the field a *T. oleracea* resembling male was found. For this specimen the period from egg to adult fly lasted 49 days, which is normal for a true *T. oleracea.* From the same egg batch a *T. czizeki* female was bred after 104 days. This female was crossed with a *T. oleracea* male from the laboratory cultures and again a *T. oleracea* female emerged, after a development of 60 days (within the range of *T. oleracea*). The remaining larvae of the second mating (9) died between 20.viii and 17.ix.1974 (about 180 days old at 20.viii.1974) (Den Hollander, unpubl.).

Even more similarities are found in the larval and pupal stages of the species concerned than in the adult flies; moreover, differences in the structure of the egg shell could not be found (Theowald, 1967, and unpubl.). This makes it worthwhile to closely compare the different species and to study their interrelationships.

MATERIALS AND METHODS

The laboratory culture of *T. oleracea* was started with a female caught in the field (male unknown) in the neighbourhood of Amsterdam on 9 August, 1973. On the 10th of August this female laid eggs from which adult flies emerged from 8 till 22 October. From these adults new cultures were started on 11, 15 and 19 October. The third generation was started on 13 December. One male and one female of this third gen-

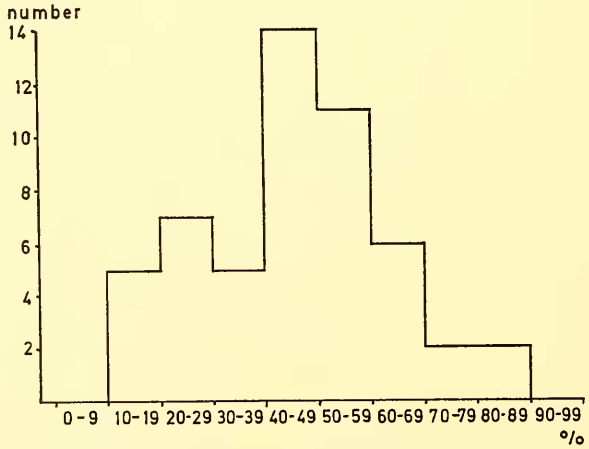

Fig. 1. The frequency distribution of the percentage of eggs from which *Tipula oleracea* larvae emerged, for 52 petri dishes with 1680 eggs

eration emerged on 11 February, 1974, and the latter produced fertile eggs on 13 February; the larvae of this fourth generation were used in the experiment. A parallel experiment was carried out with larvae descending from males and females of the next generation, which laid eggs on 29 May, 6 and 7 June. Table I presents some general information on the cultures used.

Two methods of rearing the larvae were used. In both cases females had been fertilized in the laboratory and afterwards were allowed to lay eggs in a plug of damp cotton-wool. After counting, the eggs were transferred either to a plastic box (18 × 15 × 9 cm) on wet sand or, in known quantities, on another wet plug in a plastic petri dish (diameter 9 cm, height 1.4 cm). Although the petri dishes were partitioned in three or four parts, the larvae, even the large larvae of the fourth stage, could freely move throughout the dishes. In both cases the larvae were fed with dried powdered grass (Laughlin, 1958).

The larvae emerged from the eggs on the fifth to seventh day after egg-laying (Table 2) and grew prosperously. However, the percentages of eggs from which larvae emerged proved to be quite variable (Fig. 1, Table 1). The mean percentage of hatching varied from 27 to 66% between the different cultures (Table 1), while it varied from

Table 1. General information on the laboratory cultures of *T. oleracea*

Date of start	number of eggs		% hatched		number of larvae fixed		first pupae (in days after egg laying)	last adults (in days after egg laying)
	petri dish	plastic box	petri dish	plastic box	petri dish	plastic box		
13 February	780	280	41	—	290	41	36	64
29 May	300	500	66	64	133	151	62	99
6 June	300	—	27	—	65	—	—	—
7 June	300	1200	41	47	64	161	55	102
					552	353		

Table 2. The percentage of the eggs from which larvae of *Tipula oleracea* emerged at different times after egg laying

Breeding		number of eggs	% hatched		
			after 5 days	after 6 days	after 7 days
13 February	petri dish	780	—	35	41
7 June	petri dish	300	17	41	41
7 june	plastic box	1200	13	47	47

10 to 90% in the different petri dishes, even within the same culture.

Mortality among the larvae was rather low, the greatest mortality occurring in the first two instars (14 and 8%, respectively) as well as in the second part of the fourth instar (12%) (Table 3).

Table 3. The mortality of *T. oleracea* larvae; the larvae are fixed during different periods after emergence, the % mortality refers to the total preceding period (all petri dish cultures)

Days after emergence	larval instar I	II	III	IV	total	number of eggs hatched	% mortality
1 - 7	108	10	—	—	118	137	14
8 - 14	5	86	16	—	107	138	22
15 - 24	—	2	117	43	162	206	21
25 - 35	—	—	9	94	103	129	20
36 - 48	—	—	—	46	46	68	32
					536	678	

The larvae were fixed in Faester's fluid (acetic acid 33%: alcohol 96%: glycerine: aqua dest. = 2: 35: 6: 57). When the larvae are killed in this fluid, at first the muscles totally relax, but afterwards contract to some extent. The larvae of the first experiment (started in February) were mostly measured at the day of killing (except those sampled at 8, 18, and 27 March, which were measured after 3, 2, and 6 days, respectively). The larvae of the experiments started during the end of May and the beginning of June all were measured after the experiment ended. Nevertheless, the larvae of the May/June experiment showed higher values for body length as compared to those of the March experiment. The effect of fixation in Faester's fluid is illustrated in Table 4; it shows that after one day the measurements do not change any more.

Table 4. The influence of fixation in Faester's fluid on the length of *Tipula oleracea* larvae (I: measured at the day of fixing; II—IV: 1, 2 and 3 days afterwards, respectively)

	I	II	III	IV	mean differences (mm) I-II	II-III	III-IV
body length (mm) n = 14	35.2	32.5	32.5	32.5	2.75	0.26	0.27

Table 5. The establishment of the accuracy of the measurements

	Body length (mm)	head capsule length (mm)	head capsule width (mm)
Magnification	1 ×	20 ×	40 ×
mean 1	22.6	2.79	0.83
mean 2	22.7	2.79	0.82
range 1	20.0—25.8	2.60—3.05	0.74—0.94
range 2	20.4—25.8	2.55—3.05	0.74—0.94
mean difference 1 - 2 (%)	1.12	1.03	1.55
number measured	25	25	25

The measurements (Fig. 2) were performed with a Zeiss dissectingmicroscope at a magnification of 40 × (the width of the head capsule between the antennal bases; the length of the head capsules in the first three instars; the length of the larval body during the first two days after emergence), 20 × (the length of the head capsule in the fourth larval instar; larval body length in the first instar), 10 × (the body length in the second instar). The body lengths of the larvae in the third and fourth instar were measured with vernier (0.1 mm) calliper. The accuracy of the measurements is given in Table 5.

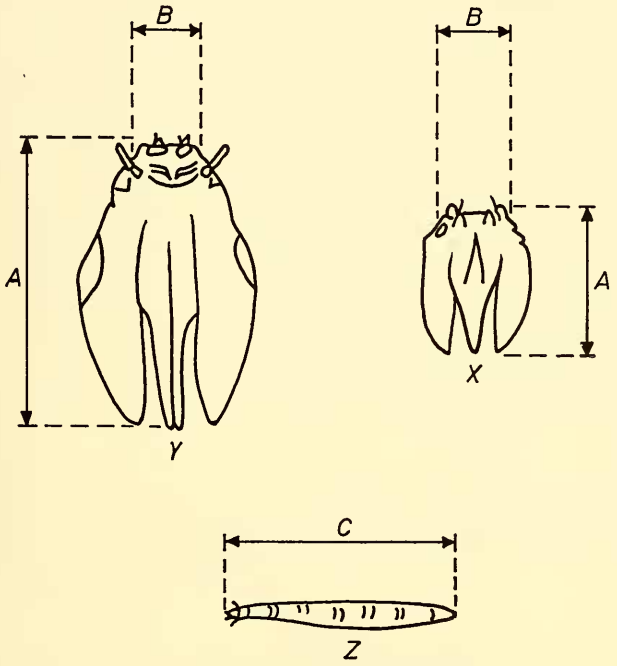

Fig. 2. The measured characters. X: first instar head capsule; Y: second, third and fourth instar head capsules; Z: larval body; A: head capsule length; B: head capsule width; C: larval body length

RESULTS

I. The durations of the larval instars

Larvae of *T. oleracea* were sampled from the laboratory cultures according to the scheme given in Table 6. This table illustrates the number of larvae fixed during the observation period as well as the occurring mortality. Besides, the fixed larvae have been distinguished as to the larval instar. Freshly moulted larvae can easily be recognized by the colour of the head capsule. Prior to moulting they are deep black whereas just after moulting the head capsules are still unsclerotized and therefore white. Thus, by following the larvae from day to day the occurrence of moulting could be easily detected. This method was used in the experiment started on 13 February. In the other experiments the width of the head capsule was used as an additional character to distinguish the larval instars (see chapter II).

Table 6. The number of *Tipula oleracea* larvae sampled at different times from the cultures. In brackets the numbers of eggs from which larvae emerged. (×): the total number of larvae emerged from the eggs amounted in these four samples to 76. (P): On this day the first pupae appeared in the culture. A: petri dish. B: plastic box culture

Culture days after egg laying	13 February		29 May		6 June	7 June		larval instars				
	A	B	A	B	A	A	B	I	II	III	IV	Total
6	13 (13)	—	—	20	—	—	—	33	—	—	—	33
7	4 (6)	—	—	—	—	—	—	4	—	—	—	4
8	10 (12)	—	18 (20)	—	—	—	—	28	—	—	—	28
9	16 (16)	—	—	—	—	—	—	16	—	—	—	16
10	13 (13)	—	15 (17)	—	—	—	—	28	—	—	—	28
12	16 (16)	—	13 (24)	—	—	—	20	22	27	—	—	49
13	12 (13)	—	—	—	—	—	—	4	8	—	—	12
14	15 (17)	—	20 (26)	20	—	—	—	2	53	—	—	55
15	7 (9)	—	—	—	—	—	—	—	7	—	—	7
16	14 (14)	—	11 (21)	—	—	—	—	—	25	—	—	25
19	15 (16)	—	13 (22)	—	—	—	20	—	16	32	—	48
20	9 (12)	—	—	—	—	—	—	—	—	9	—	9
21	—	—	9 (13)	20	—	—	—	—	1	28	—	29
22	27 (×)	—	—	—	—	—	—	—	2	25	—	27
23	13 (×)	—	16 (19)	—	—	—	—	—	—	28	1	29
26	13 (×)	—	9 (16)	—	—	—	20	—	—	25	17	42
27	22 (22)	—	—	—	—	—	—	—	—	22	—	22
28	—	—	9 (20)	20	—	17 (28)	—	—	1	10	35	46
29	18 (×)	—	—	—	—	—	—	—	—	7	11	18
30	22 (25)	—	—	—	—	—	—	—	—	8	14	22
32	—	—	—	—	16 (20)	—	—	—	—	—	16	16
33	—	20	—	—	—	12 (19)	20	—	—	2	50	52
35	8 (9)	—	—	21	—	—	—	—	—	—	29	29
36	13 (17)	(P)	—	—	—	—	—	—	—	—	13	13
39	—	—	—	—	22 (22)	—	—	—	—	—	22	22
40	10 (17)	—	—	—	—	—	21	—	—	—	31	31
42	—	21	—	20	—	14 (23)	—	—	—	—	55	55
45	—	—	—	—	18 (25)	—	—	—	—	—	18	18
46	—	—	—	—	—	—	20	—	—	—	20	20
49	—	—	—	20	—	—	—	—	—	—	20	20
52	—	—	—	—	—	5 (5)	—	—	—	—	5	5
53	—	—	—	—	9 (15)	—	—	—	—	—	9	9
54	—	—	—	—	—	—	20	—	—	—	20	20
55	—	—	—	10	—	(P)	—	—	—	—	10	10
56	—	—	—	—	—	16 (49)	—	—	—	—	16	16
59	—	—	—	—	—	—	20	—	—	—	20	20
62	—	—	—	(P)	—	—	—	—	—	—	—	—
total	290	41	133	151	65	64	161	137	140	196	432	905

Table 6 shows that the length of instar I amounts to 6 to 8 days. Within a period of three days all larvae moulted. The instar II larvae occur in the cultures for about 8 days, though they may occur in very small numbers in the cultures afterwards. Instar III larvae could be found for a period of 12 days; however, already from about 6 days after the appearance of the third instar larvae, instar IV larvae occurred in the cultures. So, the length of the third instar seems to vary. Instar IV larvae clearly get the upper hand about 10 days after the second moult. These larvae could be found in the cultures for a rather long period, a large part of which they did not show any activities. Before, the larvae could be seen creeping upon the substrate, especially early in the morning, and the results of their nocturnal activities were very clear in that the substrate had been strongly rooted up. However, just after the third moult, these activities could not be observed any more. Moreover, the larvae seemed to have moved deeper into the substrate and to stay there motionless.

The appearance of the first pupae rather varies for the various cultures. The earliest appearance occurred in the culture started at 13 February, viz., on the 36th day after egg laying and about 10 days after the start of the third moult. On the other hand, in the culture started at 29 May they appeared not before the 62nd day after egg laying, i.e. about 35 days after the beginning of the third moult. In fourteen other cultures, covering more than 500 pupae, the first pupae appeared in seven cases before the 50th day after egg laying, whereas in two cases they appeared as late as on the 71th day; the mean value amounted to 53.6 days. Therefore, considerable variations exist as to the length of the fourth larval instar in the various cultures, but also in one and the same culture: pupae generally appear over a period of about 20 days, but periods of up to 50 days do occur.

Summarizing the results it may be stated that synchronization is very strong during the egg stage as well as during both the first and second larval instar. Afterwards, synchronization decreases, so that adults appear over a considerable period, though being raised from one and the same egg batch.

II. The values of the measurements for each larval instar

Because both the body length and the length of the head capsules show intrastadial growth, it is not significant to give mean values for these characters as they only reflect the period of sampling. The width of the head capsule, measured between the antennae, on the other hand, does not show intrastadial growth, but increases suddenly just after each moult. So this character proves to be very useful for distinguishing the successive larval instars. The measurements are given in Table 7. Fig. 2 shows the characters measured in this study.

Table 7 A shows that the width of the head capsule increases from instar to instar by a factor of about 2.0. This factor seems to be somewhat higher in the first larval instar (2.1) than in the later instars (1.8). The length of the head capsule gradually increases, both within and between the successive larval instars. Discontinuities due to moulting do not exist in the distribution of the head capsule lengths. In agreement with this, the head capsules shed at moulting show values which lie in the upper part of the range of the head capsules of the former instar, as well as in the lower part of the range of the next instar. The increase in mean length of the shed head capsules over

Table 7. The values of the head capsule width (A), head capsule length (B), shed head capsule length (C) and body length (D) for *Tipula oleracea* larvae

A. The width of the head capsule (mm)

Culture	instar	mean	range	number measured
29 May	I	0.12	0.10—0.14	58
	II	0.25	0.24—0.26	66
	III	0.47	0.43—0.55	63
	IV	0.86	0.72—0.96	97
6 June	IV	0.89	0.77—0.98	65

B. The length of the head capsule (mm)

Culture/instar	I	II	III	IV
13 February	0.26—0.67	0.60—1.15	1.08—2.15	1.70—3.25
25 May	0.24—0.70	0.70—1.25	1.22—2.28	2.15—3.05
6 June				2.65—3.35
7 June	0.60—0.62	0.79—1.15	1.25—2.20	1.95—3.10
number measured	137	140	196	396

C. The length of the shed head capsules at moulting (mm) from culture 13 February

Number	instar	mean	range
68	I-II	0.64	0.60—0.70
119	II-III	1.13	1.00—1.25
52	III-IV	1.99	1.70—2.30
20	IV-pupa	2.86	2.65—3.20

D. The length of the body (mm)

Culture/instar	I	II	III	IV
13 February	1.1—5.1	3.3— 9.6	6.2—18.6	9.0—34.0
29 May	1.1—5.3	3.8—11.9	9.0—20.6	11.3—36.3
6 June				25.8—36.7
7 June	3.8—5.0	5.1—10.3	8.0—19.5	14.0—34.5
number measured	137	140	196	396

the successive moults amounts to about 1.7 (Table 7C). The largest proportional intra-instar increase in head capsule length was found during the first larval instar (× 2.9, i.e., the highest value divided by the lowest one, Table 7B), whereas these factors show about equal values in the other instars (× 2.0). The same holds true to a large extent for the body length (Table 7D). These values also gradually increase both within and between the different instars. However, some discontinuity seems to exist in the distributions due to moulting (see chapter III).

Again, growth (increase in body length) is proportionally greatest during the first larval instar (× 4.8). During the following instars the body length increases by factors amounting to 3.6, 3.3 and 4.1, respectively. Concerning the last factor one should take into account that this is realized during a much longer period than the other figures (see chapter I and Table 6).

Dyar's Law states that the larval head capsule generally grows in geometrical progression, increasing in size by a constant ratio at each moult. This rule also applies to the growth in lengths and weights and is valid in many insect species and other arthropods (Dyar, 1890; Wigglesworth, 1972; Imms, 1970). From the present data it may be concluded that Dyar's Law holds true for both the width of the head capsule (as measured in the present study) and the length of the shed head capsule. So both these characters can be considered very useful for differentiating between larval instars. On the contrary, Dyar's Law is not applicable to the length of the head capsule or body length because both show intrastadial growth. Therefore, these cannot be used as characters to distinguish the different instars or the numbers of instars, as, e.g., has been done by Lam & Webster (1972). The presence of head capsule size groups is no proof of corresponding instars, because the groups may have originated from different ovipositions or have been caused by ecological fluctuations.

Przibram's rule (Przibram & Megusar, 1912; Wigglesworth, 1972), another empirical law of growth, states that at each moult all linear dimensions increase by the ratio 1.26. However, all linear dimensions measured in the present study show much higher values of increase, whereas these values are not always constant for each moult. In general the growth-rate decreases in the successive instars.

III. The growth of larvae

The values of the measured characters are plotted against time in Fig. 3. It presents all measurements from the cultures described above, from the 6th to the 55th day after oviposition. Thus 56 samples, containing altogether 869 larvae, and taken at 34 different days have been incorporated (Table 6). Fig. 3, again, shows the discontinuous growth of the width of the head capsule. Both other measurements (body length and head capsule length) behaved in a very similar way during the observation period. The values gradually increase during the first three larval instars, and just after the third moult reach constant values, which are maintained until the end of instar IV. So growth of the larvae occurs in the first half of their life, which includes all three moults. However, during the growth period, the rate of growth gradually decreases, i.e. the proportional growth decreases with time. In addition, during the growth period, the relation between the length of the body and that of the head capsule tends to change. This becomes evident when plotting the length of the body against that of the head capsule (Fig. 4). It shows that at each moult a decrease occurs in body length, absolute, and relative to head capsule length. However, within each instar the growth of both shows to be clearly allometric.

As important differences exist between the size of male and female pupae (males are about 20% shorter), it might be assumed that these differences should already be reflected in instar IV larvae. However, analysis of the frequency distribution of the body length (189 larvae from 13 different samples) in the second part of the fourth instar (older than 37 days, Fig. 3) dit not reveal any bimodality.

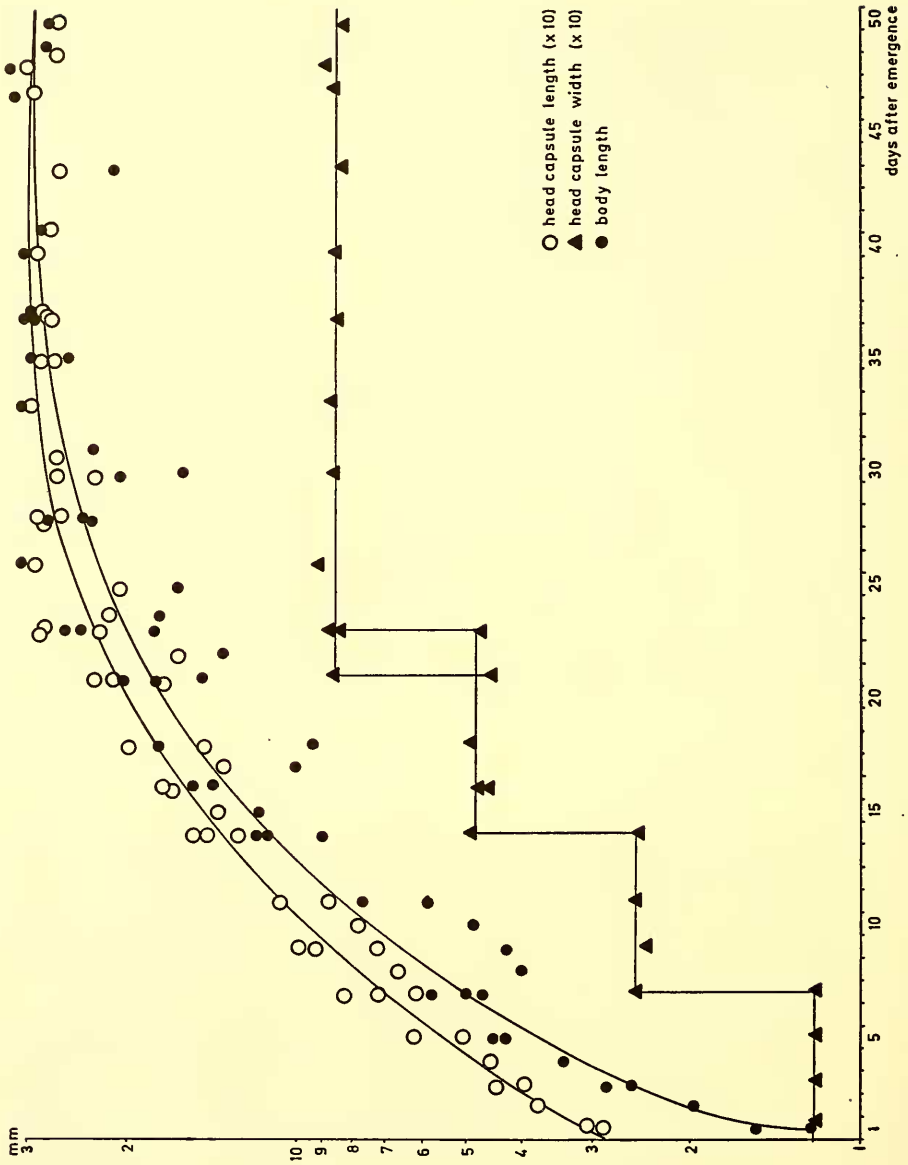

Fig. 3. The mean values for the head capsule length, head capsule width and body length during the four larval instars of *Tipula oleracea*

Fig. 4. The relation between head capsule length and body length for each of the four larval instars of *Tipula oleracea*

Nevertheless, "large" and "small" larvae can be distinguished in the cultures. From one culture (Table 8A) half of the larvae were selected at random. Both halves then were divided into three groups: yellow coloured larvae, small grey coloured larvae and large grey coloured larvae. One set of the groups was fixed in Faester's fluid, the other set was kept in order to establish the date of pupation as well as the pupal sex ratio. From another culture (Table 8B), the larvae were divided at first into two size groups, and afterwards from both samples 10 larvae were fixed. The results are given in Table 8. It shows that, in both males and females, the body shortens just before

Table 8. Differences in body length and head capsule length between *Tipula oleracea* larvae from which male, and larvae from which female pupae will arise

Culture A (about 90 days after egg-laying)	"yellow"	"small"	"large"
body length (mm)	23.5(17.0—30.8)	27.6(23.0—32.8)	31.3(26.4—34.4)
head capsule length (mm)	2.86(2.50—3.10)	2.76(2.50—3.05)	2.93(2.75—3.10)
head capsule width (mm)	0.87(0.79—0.91)	0.83(0.79—0.94)	0.89(0.79—0.96)
number fixed	18	29	28
number kept alive	21	28	29
first pupae (after days)	1	7	7
last pupae (after days)	11	25	33
total pupae	6♂, 7♀	11♂, 8♀	11♀
Culture B (56 days after egg-laying)			
body length (mm)		27.0(24.6—28.9)	30.9(27.8—35.7)
head capsule length (mm)		2.70(2.60—2.85)	3.00(2.90—3.10)
number fixed		10	10
number kept alive		24	24
first pupae (after days)		7	11
last pupae (after days)		38	33
total pupae		17♂, 1♀	17♀, 3♂

pupation. Simultaneously, the larvae become yellow. Although there is a considerable overlap, male larvae generally are smaller than female larvae, both in body and head capsule length. This overlap, as well as the differences between various cultures is responsible for the fact that the frequency distribution of the body lengths of fullgrown fourth instar larvae is not bimodal. Nevertheless, the results show that sex-differences become apparent at least at the beginning of the fourth instar. Because the differences in body length are reflected by the same differences in head capsule length, it is concluded that male larvae stop growing earlier than female larvae.

Comparison of the results of culture A with those of culture B shows that the determination of the sex in the larvae — in particular selecting the males — becomes

more difficult and less precise with advancing age. Whereas culture B was sexed just at the moment that the first pupae appeared, pupae were present in culture A for a period of about one month before the moment of sexing. From this culture already 100 ♂ and 12 ♀ had pupated in that period. In older cultures mistakes can easily be made between "yellow" larvae and "small" larvae, which may have caused the relatively large number of females produced by the "small" larvae (Table 8A).

IV. Preliminary observations on the growth of *Tipula paludosa*

Some preliminary observations were made on the size of the larvae of *T. paludosa* in the third and fourth larval instars. The larvae were obtained in two different ways. In the first place, fourth stage larvae were sampled in the field from April to August. Most of these larvae were raised to the adult stage (Den Hollander, in press); 23 of them were fixed as full grown fourth instar larvae. In the second place, *T. paludosa* larvae were bred in the laboratory from eggs laid by females which had been raised from the larvae mentioned above. Seven fourth stage larvae and 30 third stage larvae were sampled from this culture. The results are given in Table 9.

Table 9. The values (mm) for the body length, head capsule length (ranges) and head capsule width (ranges and means) of the third and fourth larval instars of *T. paludosa*. 1: larvae sampled in the field; 2: larvae bred in the laboratory; 3: summation of 1 and 2

Instar	number	body length	head capsule length	head capsule width
III	30	8.0—22.0	1.40—2.70	0.53—0.65 (0.58)
IV (1)	23	30.3—48.0	3.10—4.00	0.96—1.15 (1.04)
IV (2)	7	16.6—30.3	2.90—3.35	0.96—1.18 (1.06)
IV(3)	30	16.6—48.0	2.90—4.00	0.96—1.18 (1.04)

The first larvae emerged from the eggs after 7 days, most larvae hatching on the 8th day. After 48 days most of the larvae in the culture still were in the third instar. Thus, the developmental rate of *T. paludosa* seems to be somewhat lower as compared to that in *T. oleracea*, which probably is true for all instars.

Table 9 shows that the measurements show higher values in *T. paludosa* as compared to *T. oleracea*. This holds especially for the head capsule width, in which the ranges scarcely overlap. The overlap is considerable for the two other measurements.

Both the length of the body and the head capsule length show intrastadial growth in *T. paludosa*, and also in *T. oleracea*. Plotting the length of the body against that of the head capsule (compare Fig. 4: *T. oleracea*) again the clearly allometric growth of both measurements within each larval stage is shown. The results suggest that the regression lines of the larval instars of *T. paludosa* parallel those of *T. oleracea*. In the formula $\log y = \log a + b \log x$, which describes the relationship between the two variables, body length and head capsule length, only the y-intercept (a) differs for the different larval instars, as well as between the different species; the regression coefficient (b) probably shows the same value throughout the different larval instars and in both species. Thus, the specific differences between *T. paludosa* and *T. oleracea* only concern

the fact that the former species has longer larval head capsules (about 10%) than *T. oleracea* larvae of the same body length. These differences may be traced back to the differences in egg size between the two species (Hemmingsen & Birger Jensen, 1972). The increase of both measurements in relation to each other, seems to be similar in both species, though differences in the durations of the larval instars do occur.

DISCUSSION

Up till now many difficulties existed in recognizing the different species of the subgenus *Tipula* Linnaeus, 1758, especially in the immature stages. In adults this is also true for females. In addition, generally two or more species may be found in the same habitat together. Despite the rather distinct shape of the male genitalia, cross pairings can be achieved in the laboratory between males and females of different species, and even between those of different subgenera (e.g. *T. czizeki* and *T. luteipennis*, subgenus *Tipula* and *Platytipula*, respectively). Sometimes copulating pairs consisting of different species have been caught in the field (*T.* (*Tipula*) *paludosa* Meigen ♂ × *T.* (*Mediotipula*)? *brolemanni* Pierre ♀; Theowald, pers. comm.). This has also been observed for the species of the subgenus *Tipula* which occur in the Netherlands (cf. Introduction). The species of the subgenus *Tipula* thus show rather great similarities and close relationships. It is, therefore, very interesting to compare these species as to their biology. The present paper deals with the growth of *T. oleracea* and is a first contribution to this field of research.

The results show that the development of the larvae takes about 50—60 days under laboratory conditions (20—25° C), half of this period is covered by the fourth instar. Laughlin (1958, 1960) found that the complete life cycle of *T. oleracea* at 21° C takes an average of 11—12 weeks, which is in accordance with the present results. He also found the extremely wide variation in the duration of the fourth instar; however, his figures show that not only the length of the fourth instar varies, but also that of the other instars. The present results show that synchronization is rather strong from the egg stage up to the third moult. Variations in the total length of the larval period are, therefore, mainly due to variations in the length of the fourth instar.

Laughlin (1960), measuring the weight of the larvae, showed that growth is exponential during the first three instars whereas a constant daily weight increase occurs in the fourth instar. Just before pupation the weight decreases with about 50%. To the contrary, the present study reveals that the length of the body as well as the length of the head capsule do show exponential growth, though the relationships between $\log_{10}$ length and time are not linear, i.e., the growth rate decreases in the successive larval instars. Moreover, only during the first ten days of the fourth instar these measures increase in value but later remain at a constant level. Thus, about 75% of the increase in length occurs in the first half of the larval period.

Rodriguez & Maldonado (1974), studying the praying mantis *Stagmatoptera biocellata*, also found that the growth rate decreased in the successive instars. In addition they found that Dyar's law, as well as Przibram's rule, did not apply to this species, as was equally the case in the present study. A constant body size, measured in body weight, was also found in the last larval instar of *Calliphora* (Vijverberg, 1974). In this species a significant growth retardation of the larvae occurred after about 25—30% of the last larval instar had elapsed. During that period important changes in many

processes occur in Diptera e.g., in growth, behaviour, hormone titres, etc. (survey in Vijverberg, 1974).

The above phenomena are very interesting when compared to the growth of *T. paludosa*. Whereas *T. oleracea* completes two generations a year, *T. paludosa* has an annual life cycle. However, both species overwinter as third instar larvae and moult to the fourth instar during early spring (April). Thus, growth of *T. paludosa* equals that of *T. oleracea* which begins to grow during the same period (August, September). However, adults of *Tipula oleracea* emerge in May but those of *Tipula paludosa* only as late as August. So, the differences in phenology between both species are the results of differences in the length of the fourth instar. Both species show very little activities as fourth instar larvae and, in addition, there is little or no growth during this period (De Jong, 1925; Laughlin, 1967; Den Hollander, in press).

On the basis of the above data the hypothesis may be put forward that *T. oleracea* and *T. paludosa* have evolved under different climatological conditions, *T. oleracea* under warm and *T. paludosa* under cold conditions. Under colder conditions there may then have been a selection pressure towards longer fourth instar duration, perhaps because the eggs and/or the young larvae could not develop in early summer conditions when the surface became inundated by melting water. Both eggs and larvae of *T. paludosa* as well as of *T. oleracea* are susceptible to soil flooding (Meats, 1970, 1972). Other data in favour of this hypothesis are the following: the emergence of adults of *T. paludosa* is much more synchronized than in *T. oleracea*, and in *T. paludosa* females the wings are shortened in relation to body length which is a character which occurs especially in organisms adapted to colder climates (Beyers, 1969). It might be very worthwhile, in this context, to select strains of *T. oleracea* with the longer durations of the fourth instar and compare these strains to *T. paludosa*.

The data presented here reveal a supplementary character to distinguish larvae of *T. paludosa* and *T. oleracea*. Brindle (1959) and Theowald (1967) mention differences in the shape of the ventral papillae of the anal segments. However, this character is rather variable, and besides, it is not distinct but gradual. However, the width of the head capsule, measured between the antennal bases can be established easily and with great accuracy. The mean values for the different instars lie on a straight line when the $\log_{10}$ of them is plotted against the larval instar, both in *T. oleracea* and in *T. paludosa* (Table 7; Hemmingsen, 1965). The results of the measurements in *T. paludosa* showed mean values of 0.58 for the third larval stage and 1.04 for the fourth larval stage (Table 9). From these values, and from the assumption that the lines for both species run parallel (which is the case in *T. saginata* Bergroth and *T. paludosa*, see Hemmingsen, 1965), the mean values for the first two instars of *T. paludosa* can be deduced (0.16 and 0.30 mm, respectively). Because of the rather slight variation in this character, one may expect these values to be useful to distinguish between *T. oleracea* and *T. paludosa*. Besides, slight differences in colour exist between the larvae of both species (*T. paludosa* is more yellowish, *T. oleracea* more greyish), as well as in size (*T. paludosa* is bigger). However, both characters change just prior to pupation, i.e., *T. oleracea* larvae then become also yellowish, whereas the larvae of both species become smaller.

Unfortunately, it seems very difficult to distinguish between the larvae of *T. czizeki* and *T. oleracea*. Some preliminary measurements do not show any differences between the larvae of both species; in addition, they also are coloured identically.

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